

# Investigation into the Antibacterial and Freshness Preservation Efficacy of Dry Porous Bags of Biomass Ash from Agricultural Residues

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Biomass ash from agricultural residues, which is typically discarded and causing waste and pollution, was evaluated in tea-bag form as a natural food preservative in this study, addressing research gaps on its effects and safety. *In vitro* tests on six biomass ash extracts demonstrated significant antibacterial activity. Specifically, the golden leaf fine branch camellia extract exhibited the highest efficacy against *Staphylococcus aureus* and *Alternaria brassicicola*, with an inhibition zone of 21 mm and an antibacterial efficiency of 70.2%. Fruit storage tests confirmed its preservative ability. Golden leaf fine branch camellia twig and leaves ash exhibited the most effective weight loss reduction for potatoes, corn stalks ash was found to be optimal for green peppers, rice straw ash offered the most effective preservation for sugar oranges, and soybean straw ash yielded favorable outcomes for mangoes. Safety evaluations revealed no genotoxicity, with lead content below the 0.2 mg/kg safety threshold and the micronucleus tests within safe limits. Elemental analysis detected high potassium content (103.127 g/kg), while SEM and FTIR revealed a porous structure and functional groups that may contribute to its antibacterial and preservative properties. In conclusion, biomass ash has strong antimicrobial and preservative properties, is non-genotoxic, and shows great potential as a natural food preservative, providing a new avenue for biomass ash resource utilization.

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## INTRODUCTION

Biomass ash, derived from the combustion of diverse biomass materials such as wood, agricultural waste, and other organic residues, is a byproduct with significant potential for resource utilization (Heppner and Livney 2024). It is estimated that China has approximately 600 million tons of biomass available annually for energy production, with an annual ash residue production of over 40 million tons (Li *et al.* 2022). However, the utilization percentage of biomass ash varies significantly worldwide. In some developed countries, such as Germany, Japan, and Denmark, the resource utilization of biomass ash residues ranges from 70% to 90% (Knapp and Insam 2011). In contrast, the comprehensive utilization of biomass ash in China is relatively low, primarily due to insufficient understanding of its characteristics and potential applications. This substantial amount of biomass ash not only exerts environmental pressure but also presents opportunities for resource utilization. Currently, most biomass ash is treated as waste and disposed of or

landfilled, leading to severe resource waste and potential environmental pollution issues (Smith *et al.* 2022). Therefore, it is imperative to explore means to promote the comprehensive utilization of biomass ash and reduce its environmental impact.

Recent studies have shown that biomass ash is a highly versatile resource with diverse applications. It can be used in road construction to strengthen concrete and harden surfaces (Odzijewicz *et al.* 2022). It also can be used in the reclamation of post-coal-mining lands. Industrial biomass combustion fly ash is being explored as a zeolite precursor for CO<sub>2</sub> adsorption (Petrovic *et al.* 2024), and biomass ash also finds uses in environmental protection, zeolite synthesis, rare earth metal recovery, and plastic production (Rouhani *et al.* 2024). In the agricultural sector, crop straw ash is widely used to boost soil fertility, control pests and diseases, and promote seedling growth (Smith 2023). It's even used in foods such as alkaline zongzi and gray water rice cakes to improve texture and extend shelf life (Smith 2023). Biomass ash, especially from agricultural residues such as rice husk, has unique features. Compared with traditional environmental remediation materials such as activated carbon, its nano-porous structure allows it to effectively adsorb heavy metals and organic pollutants, and as a by-product, it's more sustainable in cost and resource utilization (Moayedi *et al.* 2019). During the thermal degradation processes of pyrolysis and combustion, the alkali and alkaline earth metals (AAEMs) in biomass ash act as catalysts. They influence bio-oil and biochar yields and properties, unlike some inert additives. However, AAEMs in ash can react with SiO<sub>2</sub> and Al<sub>2</sub>O<sub>3</sub> during thermochemical conversions, causing slagging, fouling, and corrosion, which reduces thermal efficiency and raises maintenance costs (Voshell *et al.* 2018). In construction, as a supplementary cementitious material (SCM), biomass ash can enhance concrete strength and durability. Its different chemical composition compared to traditional SCMs such as fly ash might offer advantages in resisting specific chemical attacks (Esteves *et al.* 2012). In agriculture, it serves as a natural soil amendment, rich in nutrients like Ca, Mg, K, and P, and helpful for adjusting soil pH and improving water retention, contributing to sustainable agriculture compared to synthetic fertilizers (Khan *et al.* 2024). Despite its benefits, improper disposal of biomass ash can pose environmental risks. Although it contains low levels of heavy metals and PAHs and is suitable for agricultural use (Silva *et al.* 2019), proper management is still crucial.

Food supply and food safety are significant public health issues globally. Food is prone to spoilage and deterioration during production, transportation, and storage due to environmental factors (Gokoglu 2019), harmful microorganisms, and related toxins, leading to food waste and economic loss, and even posing health risks, causing food poisoning and foodborne diseases (Lam *et al.* 2013; Smith *et al.* 2022). Therefore, adding appropriate preservatives to food has become an indispensable part of processed food production. Food preservatives include chemical preservatives and natural preservatives. Chemical preservatives, such as sorbic acid and benzoic acid, pose long-term safety risks such as carcinogenesis and teratogenesis (Chaleshtori *et al.* 2018). In contrast, natural plant-derived preservatives have gained attention due to their green, natural, safe, non-toxic, and broad-spectrum antimicrobial advantages (Chaleshtori *et al.* 2018). Recent studies have shown that biomass ash can be used as a natural antimicrobial agent, with significant potential for antibacterial and antifungal activities (Liu 2016; Bang-Andreasen *et al.* 2017). Biomass ash has been shown to effectively inhibit the growth of pathogens and viruses, making it a promising candidate for disinfectant applications (Liu 2016). Additionally, biomass ash has been explored for its potential in food packaging, with studies demonstrating its ability to enhance the shelf life of perishable items such as fruits and

vegetables (Dang *et al.* 2025; Liang *et al.* 2025). However, the physicochemical properties of biomass ash from different sources vary, leading to differences in their preservative and freshness-maintaining effects. Currently, there is limited research on the preservative and freshness-maintaining effects of biomass ash, with few reports on its safety issues (Odzijewicz *et al.* 2022; Schlupp *et al.* 2024).

Specifically, biomass ash has various application methods in the preservation of fruits and vegetables, including coating, packaging, and direct application. In this study, the form of “packaging” is adopted to prepare biomass ash bags, exploring its ability to inhibit microbial growth and maintain freshness. This study explored the potential of biomass ash from agricultural residues as a natural food preservative. The antibacterial and freshness-preserving properties of biomass ash extracts derived from different sources were evaluated, including rice straw, soybean straw, corn stalks, tea stems, wicker branches and leaves, and golden leaf fine branch camellia twig and leaves. This study differs from previous ones by focusing on the comprehensive evaluation of biomass ash's antibacterial and preservative effects, as well as its safety profile, using a variety of biomass sources and experimental methods, and evaluated its preservative effect on different fruits and vegetables. This research aimed to provide experimental evidence for the development of biomass ash as a safe and effective plant-derived preservative, addressing the gap in current literature regarding the application of biomass ash in food preservation.

## EXPERIMENTAL

### Materials and Methods

#### *Reagents*

Potato Dextrose Agar (PDA) medium (Hangzhou Microbial Reagent Co., Ltd., 20191204-00), Müller-Hinton agar (M-H) medium (Hangzhou Binhe Microbial Reagent Co., Ltd., 180502), hydrolyzed casein agar medium (Hangzhou Binhe Microbial Reagent Co., Ltd., 180502), sodium, magnesium, calcium, potassium, lead standard solutions (Shanghai Maclin Biochemical Technology Co., Ltd.)

#### *Preparation of biomass ash extract*

To prepare the biomass ash extract, six different types of plant biomass ash were selected: rice straw (Str), soybean straw (Soy), corn stalks (Cor), tea stems (Tea), wicker branches and leaves (Wic), and golden leaf fine branch camellia twig and leaves (Gol). For each type, 100 grams of biomass ash was mixed with 1000 milliliters of distilled water and allowed to incubate overnight to ensure thorough soaking. This mixture was then centrifuged at 4000 revolutions per minute (rpm) for 10 minutes to separate the solid particles from the liquid. The supernatant, which contained the extracted components, was carefully removed, and the centrifugation process was repeated two additional times with the remaining precipitate to ensure complete extraction. The collected supernatants were then combined and reduced to a final volume of 100 milliliters using a rotary evaporator, resulting in a concentrated biomass ash extract with a concentration of 1 gram per milliliter, ready for use in subsequent experiments (Romanowska-Duda *et al.* 2024).

### *Determination of antibacterial activity of biomass ash*

The disc diffusion method (Boukhatem *et al.* 2020) was used to assess the antibacterial activity of biomass ash extracts against *Staphylococcus aureus* (*S. aureus*, ATCC25923), *Escherichia coli* (*E. coli*, ATCC25922), *Salmonella* (*S. enterica*, ATCC14028), and *Bacillus cereus* (*B. cereus*, ATCC14579). The diameters of the resulting inhibition zones were measured to gauge the antibacterial efficacy. The microdilution method (Wang *et al.* 2020) was used to prepare a gradient of biomass ash concentrations in MH broth, which served as the solvent. MIC microplate determination assays with MH broth were used as a negative control. From these assays, the minimum inhibitory concentration (MIC) of biomass ash against the bacteria was calculated, and the minimum bactericidal concentration (MBC) was determined based on the MIC results. To evaluate the inhibitory impact of biomass ash extracts on fungi, the treatments were tested against *Alternaria brassicicola* (NSF-40), *Alternaria alternata var. tenuis* (NSF-47), *Alternaria porri* (NSF-59), and *Alternaria alternata f. sp. nicotianae* (NSF-113), using a mycelial growth rate method for the study of antifungal activity (Shen *et al.* 2013).

### *Study on the effect of biomass ash on food preservation*

A “biomass ash bag” refers to a porous bag filled with biomass ash. This approach was used in the present experiments to encapsulate fruits and vegetables for preservation purposes. The biomass ash was collected and spread out in a thin layer to dry completely. This process ensured that the ash was free of excessive moisture, which could affect its preservation properties. The dried ash was then ground into a fine powder using a mortar and pestle or a grinding machine to ensure uniformity and ease of filling into the bags. Porous bags were selected to allow for gas exchange while preventing the ash from leaking out. These bags are typically made from materials like breathable fabric or mesh that do not react with the ash or the fruits and vegetables. The ground biomass ash was then carefully filled into the porous bags. The amount of ash used varies depending on the size of the bag and the volume of produce to be encapsulated.

Potatoes, green peppers, sugar oranges, and mangoes with highly similar characteristics were placed adjacent to “biomass ash tea bags”, within a sealed environment. This arrangement allowed the ash within the bags to remain in contact with the produce while ensuring that the entire setting was enclosed to potentially enhance its preservative effects. The control group without biomass ash bags was the baseline for comparison. The weight loss was monitored at intervals of every 2 days. The results were obtained by averaging the measurements from replicate trials.

### *Elemental analysis*

Approximately 0.2 g of the biomass ash sample was placed into a digestion tube, and 6 mL of nitric acid was added. The mixture was subjected to a graphite digestion system (Model: SH220N, Jinan Haineng Instrument Co., Ltd.) for heating and digestion. Once the sample was reduced to a colorless or faintly yellow solution, it was diluted 100-fold with deionized water. Concurrently, a mixed standard solution was prepared by incorporating standard samples of potassium, calcium, sodium, and magnesium. A calibration curve was constructed, and an inductively coupled plasma (ICP) spectrometer was used to quantify the elemental composition of the biomass ash from the test solution.

### *Scanning electron microscopy*

A modest quantity of desiccated biomass ash powder was positioned carefully near the center of the sample disk on a tape. Using a rubber bulb, air was blown gently outward in a radial direction from the center towards the sample disk to ensure an even dispersion of the powder across the tape. To finalize the sample preparation, a layer of gold sputtering was applied. The prepared sample was mounted for scanning electron microscopy analysis (SEM) (Dang *et al.* 2023; Liang *et al.* 2020).

### *FTIR infrared spectrum*

1 mg of the sample and 100 mg of potassium bromide were blended uniformly. The mixture was placed into a tablet press, and pressure of 12 to 15 tons was applied for a duration of 15 seconds. The compacted sample was placed in the sample holder, then inserted into the sample chamber of the infrared spectrometer. Each sample was measured in triplicate to ensure accuracy.

The parameters for the infrared scans were as follows: 32 scans per sample, a resolution of  $4\text{ cm}^{-1}$ , and a scanning range spanning from  $4000$  to  $400\text{ cm}^{-1}$ . With potassium bromide serving as the background, the spectrometer automatically corrected for interference from  $\text{H}_2\text{O}$  and  $\text{CO}_2$  during the scanning process. The raw infrared spectrum was converted to absorbance, automatically adjusted for the background baseline, and normalized to yield the standard infrared spectrum.

### *Safety evaluation of biomass ash*

Considering the presence of heavy metals in biomass ash, it is crucial to assess the extent to which lead from the biomass ash may transfer to food products. The present study aimed to ensure that the lead content in biomass ash used for preserving fruits and vegetables fell within the acceptable limits set by national food safety regulations. This evaluation is essential for safeguarding consumer health and maintaining the integrity of food safety standards.

A lead standard solution was prepared. A graphite furnace served as the atomizer to detect and plot the standard curve using an atomic absorption spectrophotometer (Model: TAS-990, Beijing Puxi General Instrument Co., Ltd.). The next step was to weigh out 0.10 grams of biomass ash to prepare the test solution, and after detection, the lead content in the test solution was determined based on the standard curve. The genotoxicity of the biomass ash was assessed using the micronucleus test method (Gong and Xu 2018). First, 60 uniformly sized broad bean seeds were soaked for 24 h, and then they were allowed to germinate for an additional 48 h. Once the primary roots grew to a length of 1 to 2 cm, 4 seeds from each group were chosen, and the root tips were immersed in the leaching solution.

Distilled water was the negative control, and a  $\text{Pb}(\text{NO}_3)_2$  solution was the positive control. The soaking process was repeated three times, followed by an 8-h recovery period. Paraffin embedding and sectioning techniques were used to observe the meristematic layer cells at the root tip, and the number of micronucleated cells and the micronucleus rate were calculated.

### *Data processing and analysis*

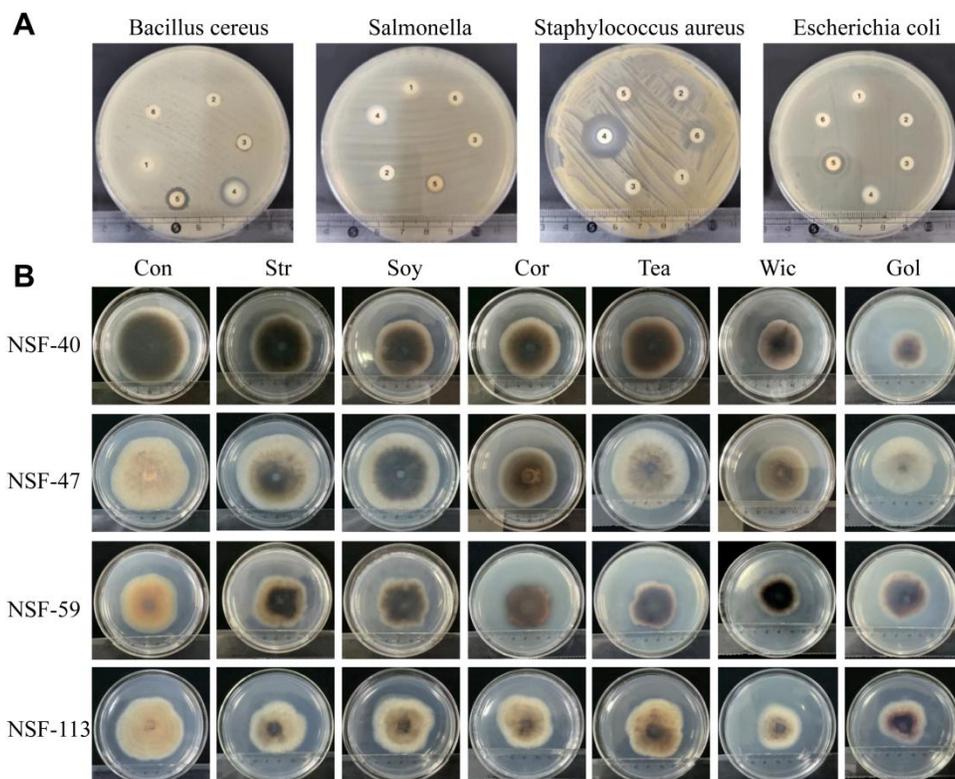
Experimental data were meticulously analyzed using SPSS 17.0 software in tandem with GraphPad Prism. A comprehensive suite of variance analyses was performed. This encompassed univariate analysis of variance (ANOVA) supplemented by Duncan's

multiple range test, as well as two-way analysis of variance. The results are presented in the form of the mean  $\pm$  standard deviation ( $\bar{x} \pm s$ ). A P - value of less than 0.05 is indicative of a statistically significant difference, while a P - value of less than 0.01 implies a highly significant difference.

## RESULTS AND DISCUSSION

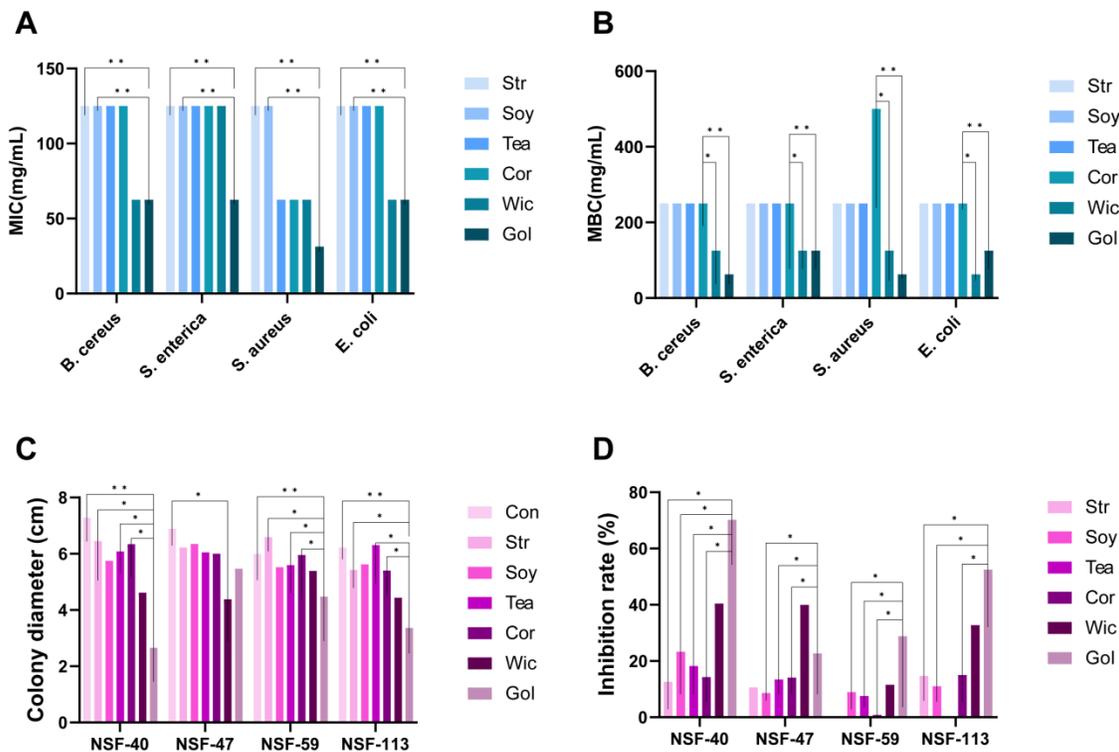
### Determination of Antibacterial Activity of Biomass Ash

As can be seen from Fig. 1, the biomass ash extracts exhibited varying degrees of antibacterial activity against the tested bacterial strains, with significant differences in their overall antibacterial performance. As shown in Fig. 1-A and Fig. 2-A&B, the golden leaf fine branch Camellia group demonstrated the strongest antibacterial effect, with the highest activity against *Staphylococcus aureus*, exhibiting an inhibition zone diameter of 21 mm, MIC value of 31.2 mg/mL ( $P < 0.01$ ), and MBC value of 62.5 mg/mL ( $P < 0.01$ ). In contrast, its antibacterial activity against *Escherichia coli* and *Salmonella* was relatively weaker, with an inhibition zone diameter of only 10.3 mm and an MIC of 62.5 mg/mL ( $P < 0.01$ ), and MBC value of 125 mg/mL ( $P < 0.01$ ). As illustrated in Fig. 1-B and Fig. 2-C&D, the golden leaf fine branch camellia group exhibited remarkable antibacterial efficacy against NSF-40, characterized by a colony diameter of merely 2.65 cm and an antibacterial effectiveness of 70.2% ( $P < 0.05$ ). Additionally, this group demonstrated a substantial antibacterial effect against NSF-113, with a colony diameter of 3.36 cm and an antibacterial effectiveness of 52.5% ( $P < 0.05$ ).



**Fig. 1.** *In vitro* antimicrobial activity of biomass ash extracts. (A) Antibacterial activity, and (B) antifungal activity of biomass ash extracts from different plants against fungi is NSF-40, NSF-47, NSF-59, and NSF-113; “Con” stands for “Control”.

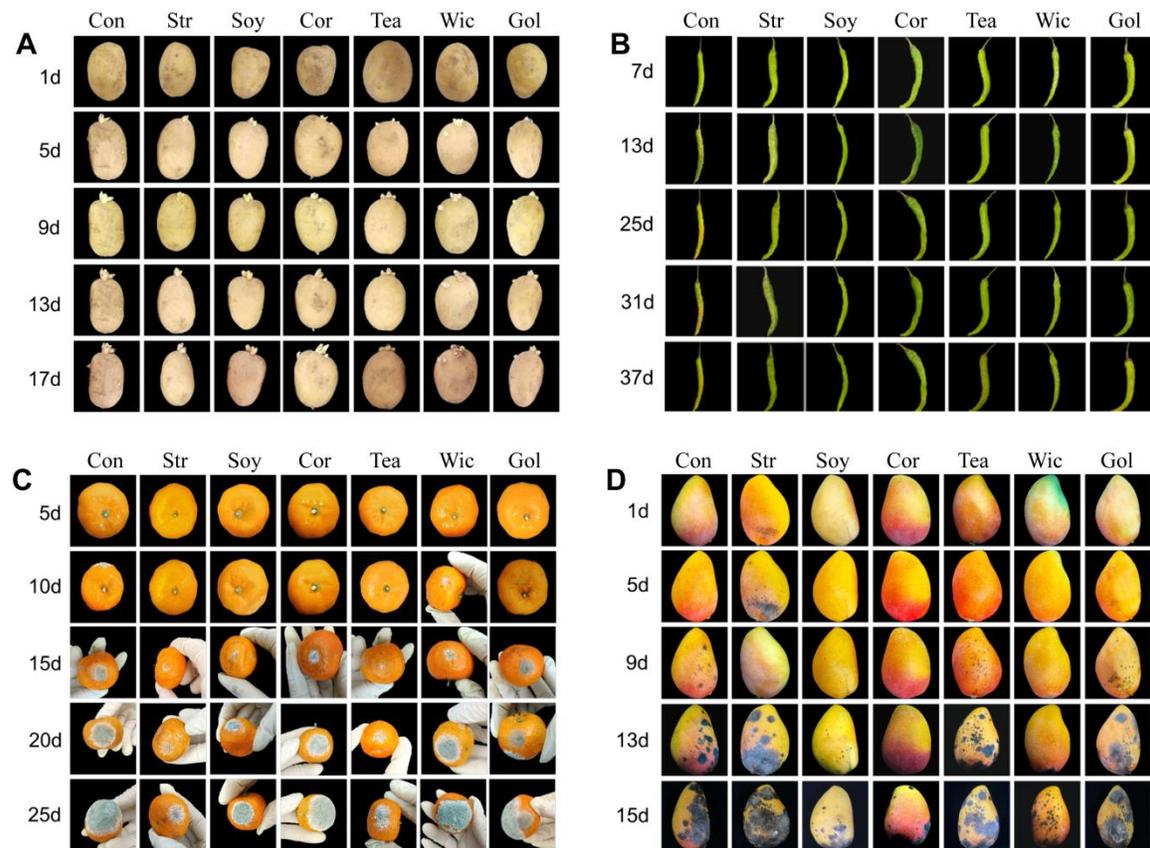
As the concentration of biomass ash increased, the inhibitory effect on the four types of fungi became more pronounced, indicating a dose-dependent antibacterial action. In summary, biomass ash demonstrates strong antibacterial properties, making it a potential candidate for the development of disinfectants and sterilizing agents.



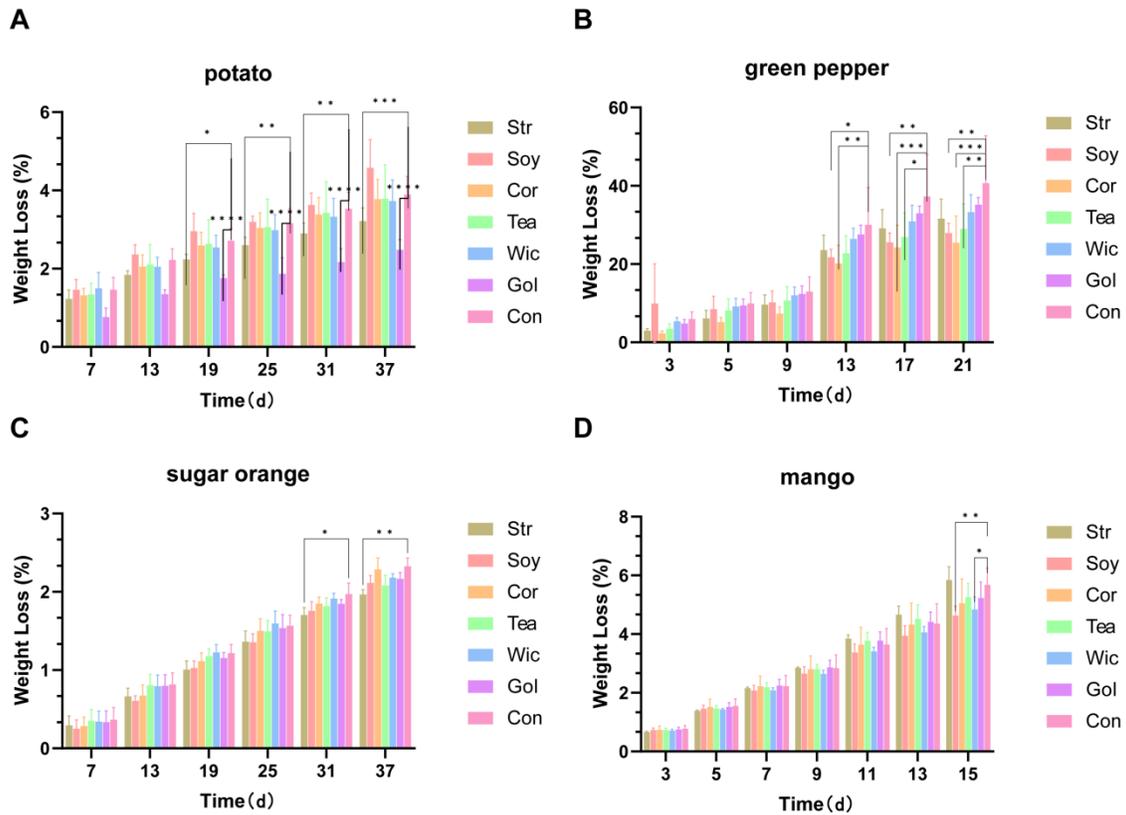
**Fig. 2.** *In vitro* antimicrobial activity of biomass ash extracts. (A) MIC antibacterial test results, (B) MBC antibacterial test results, (C) colony diameters of fungi treated with extracts, and (D) inhibition rates of biomass ash extracts against fungi; “Con” stands for “Control”.

### Study on the Effect of Biomass Ash on Food Preservation

As presented in Fig. 4-A&B, compared to the Con group, the Str and Gol groups showed significantly less weight loss in potatoes ( $P < 0.001$ ,  $P < 0.0001$ ), highlighting their greater effectiveness in potato preservation. On the flip side, the green peppers in the Con group experienced substantially more weight loss than those in the other treatment groups. The Cor group was remarkable for having the most significant preservation effect, reducing weight loss in green peppers ( $P < 0.001$ ). The Soy and Tea groups also showed beneficial effects ( $P < 0.01$ ). Figures 3-C and 4-C indicate that, after 5 days of storage, the extent of decay of sugar oranges was quite consistent across all groups. But after 25 days, the extent of decay of the Con group rose most rapidly. In contrast, the Str group has the slowest decay rate, leading to the least weight loss among all groups ( $P < 0.01$ ). Figure 4-D shows that the Soy and Wic groups effectively slowed down the increase in mango weight loss ( $P < 0.01$ ,  $P < 0.05$ ). In summary, the ash from golden leaf fine branch camellia twigs and leaves was the most effective in reducing weight loss for potatoes. Corn stalk ash was the best for green peppers. Rice straw ash provided the most effective preservation for sugar oranges. Soybean straw ash gave favorable results for mangoes.



**Fig. 3.** The impact of different plant biomass ash (straw group, soybean group, corn group, tea stem group, wicker group, golden leaf fine branch camellia group) extracts on the appearance of food storage and preservation of (A) potatoes, (B) green peppers, (C) sugar oranges, and (D) mangos; “Con” stands for “Control”.



**Fig. 4.** The influence of different plant biomass ash (straw group, soybean group, corn group, tea stem group, wicker group, golden leaf fine branch camellia group) extracts on the weight loss of food storage and preservation of (A) potatoes, (B) green peppers, (C) sugar oranges, and (D) mangos; “Con” stands for “Control”.

### Physical and Chemical Properties and Morphological Characteristics of Biomass Ash

#### Elemental analysis

Table 1 clearly shows that the nutrient content differed among the biomass ashes from various plants. All six types of biomass ashes were high in potassium, and soybean biomass ash had the highest potassium content, reaching 103 g/kg. Moreover, they contained substantial amounts of trace elements including calcium, magnesium, and sodium. The biomass ash of Golden Leaf Fine Branch Camellia had the highest calcium content, whereas soybean biomass ash had the highest levels of magnesium and sodium.

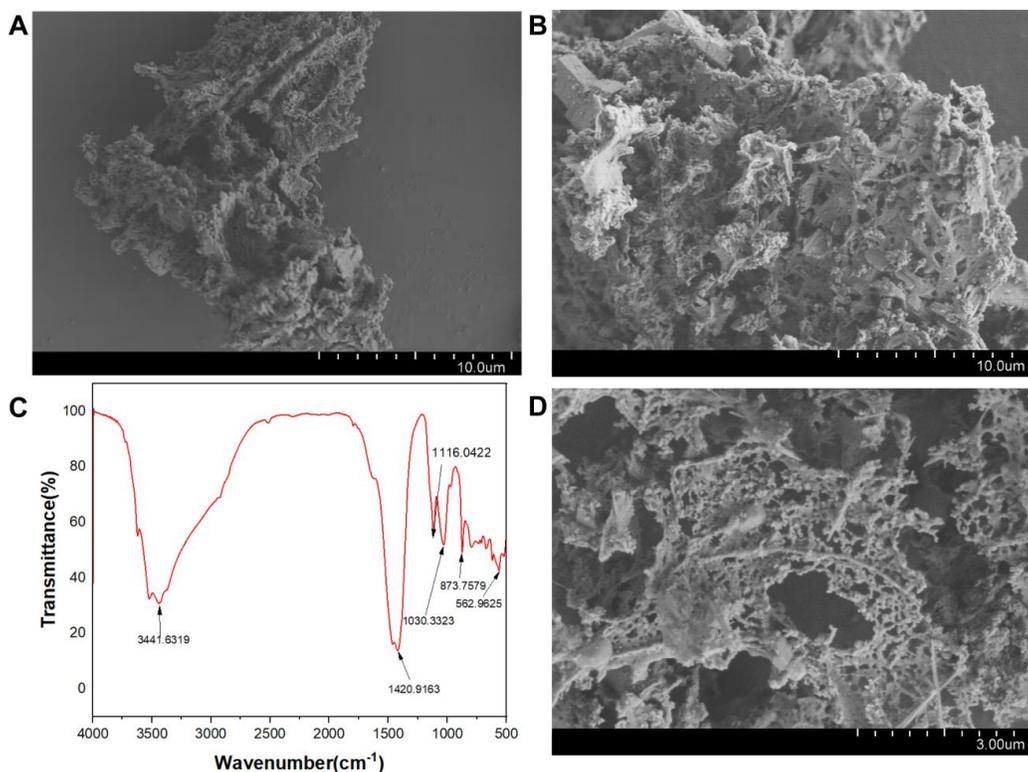
**Table 1.** The Content of Common Elements in Biomass Ash from Different Plants

Groups	Element Content (g/kg)			
	Potassium	Calcium	Magnesium	Sodium
Str	14.468	5.576	1.400	1.174
Soy	103.127	30.449	30.416	6.823
Cor	28.110	4.045	4.634	1.953
Tea	52.701	53.808	16.666	6.619
Wic	100.718	44.424	5.246	3.917
Gol	52.701	53.816	17.436	5.800

### Scanning electron microscopy detection and FTIR analysis

As depicted in Fig. 5, in images A and B under low magnification, the biomass ash presents an irregular and loose morphology. Conversely, in the electron microscope image D at high magnification, the surface appears uneven, featuring pits and intergranular spaces. Such a rough surface and the interstitial structure might confer adsorptive properties to the biomass ash (Sharma *et al.* 2017).

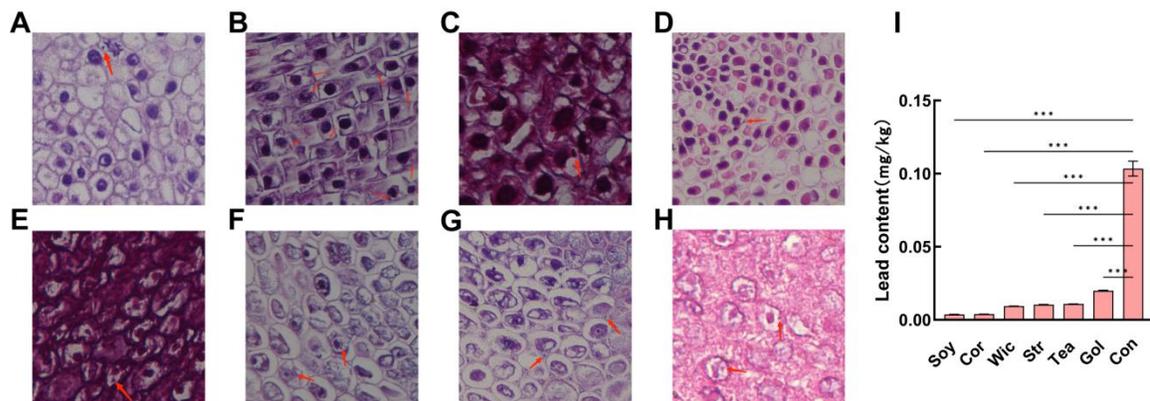
In Fig. 5-C, the FTIR (Fourier Transform Infrared Spectroscopy) analysis results highlight a pronounced absorption band near  $3441\text{ cm}^{-1}$ , which is likely indicative of O-H bonds. Characteristic peaks associated with biochar are commonly observed at approximately  $1600\text{ cm}^{-1}$ , corresponding to the aromatic C=C stretching vibrations, and within the range of  $1000$  to  $1300\text{ cm}^{-1}$ , corresponding to the C-O stretching vibrations. The graph displays noticeable peaks at  $1116\text{ cm}^{-1}$ ,  $873\text{ cm}^{-1}$ , and  $562\text{ cm}^{-1}$ , which are likely indicative of inorganic constituents. While the absence of a distinct peak at  $1600\text{ cm}^{-1}$  in the graph suggests a lower presence of aromatic C=C stretching vibrations, it does not conclusively dismiss the potential transformation of some organic materials into biochar. The strong absorption peak near  $1420\text{ cm}^{-1}$  suggests that the biomass ash contained a significant amount of alkyl, alkene, methoxy, and other group structures. The strong absorption peak near  $1030\text{ cm}^{-1}$  is related to the presence of a large amount of alcohols, carbohydrates, and other such structures on the surface. The characteristic absorption bands for aromatic groups were generally found around  $1600\text{ cm}^{-1}$ , yet the analysis did not reveal any pronounced peaks in this region, implying that the biomass ash may have contained a relatively low concentration of aromatic groups. Nonetheless, the peak at  $873\text{ cm}^{-1}$  could be associated with the C-H bending vibrations within aromatic rings, hinting at the possible presence of aromatic compounds in the sample.



**Fig. 5.** SEM and FTIR analysis (A, B, D: Scanning electron microscopy image of biomass ash; C: Infrared spectrum of biomass ash)

## Safety Evaluation of Biomass Ash

Figure 6 presents the distinct variations in lead content among the different types of biomass ash. The levels of lead content are ranked as follows: Golden Leaf Fine Branch Camellia Group > Tea Stem Group > Rice Straw Group > Wicker Group > Corn Group > Soybean Group, with the minimum content being 0.0037 mg/kg ( $P < 0.001$ ), the maximum content being 0.0199 mg/kg ( $P < 0.001$ ). Figures 6-A to 6-H and Table 2 indicate that the micronucleus percentage in fava bean root tip cells was below 10‰, and the pollution index was less than 1.5. These findings suggest that the six types of biomass ashes lacked mutagenic potential and did not pose a risk to the fava bean root tip cells. There is no evidence of teratogenic potential provided, and it can be tentatively concluded that biomass ash does not have mutagenic potential *in vivo* and is not genotoxic.



**Fig. 6.** Safety assessment of biomass ash from different plants. (A-H-Root Tip Cell Observation Results Diagram; A: Negative Control Group; B: Positive Control Group; C: Straw Group; D: Soybean Group; E: Corn Group; F: Tea Stem Group; G: Wicker Group; H: Golden Leaf Fine Branch Camellia Group; I: Lead content in biomass ash from different plants); “Con” stands for “Control”.

**Table 2.** Number of Micronuclei, Percentage and Contamination Degree of Root Tip Cells Induced by Biomass Ash from Different Plants

Groups	Total Cellular Score	Micronuclei Number	Micronuclei Per Cent	Pollution Level
Negative Control	4884	14	2.86	non-pollution
Positive Control	4989	69	13.85	Heavy pollution
Str	5155	21	4.08	non-pollution
Soy	5285	22	4.18	non-pollution
Cor	5077	21	4.14	non-pollution
Tea	4849	15	3.09	non-pollution
Wic	5195	22	4.24	non-pollution
Gol	5198	17	3.24	non-pollution

## DISCUSSION

As the ethos of safe and healthy consumption takes root, consumers are becoming increasingly concerned with the health and environmental implications of food production and processing. This trend poses significant challenges to the food industry, particularly in the case of highly perishable items such as fruits and vegetables, where the need for food preservatives is on the rise (Mesías *et al.* 2021). Natural plant-derived preservatives, with

their safety and minimal side effects, are emerging as a promising alternative to conventional preservatives (Smith *et al.* 2022). A wide variety of plant-derived natural preservatives are available, including phenols, flavonoids, aldehydes, and essential oils (Negip 2012). Common examples of these natural preservatives include tea polyphenols, essential oils, and garlic extract (Doe *et al.* 2023). The addition of xanthan gum containing 1 mg/mL of ascorbic acid or citric acid can effectively inhibit microbial activity in grapes, thereby protecting the chemical composition, color, antioxidant properties, and texture of the grapes from damage (Negip 2012). Furthermore, studies by Shehabeldine (2023) and Ooi (2006) have separately identified that clove extract and extracts from *Rabdosia rubescens* (Donglingcao) exhibit strong inhibitory effects against *Penicillium citrinum*, *Botrytis cinerea*, and *Aspergillus glaucus*. Thus plant-derived natural preservatives are regarded as potential natural preservatives, showing excellent antimicrobial activity against foodborne pathogens (Liu *et al.* 2020). Biomass ash, a byproduct of plant branch combustion, not only maintains its natural alkalinity but also retains or contains specific active substances, demonstrating effective bacteriostatic properties (Bang-Andreasen *et al.* 2017). Research has revealed that biomass ash is potent in eliminating pathogens and viruses, making it a widely utilized disinfectant ingredient in rural settings (Liu 2016).

Building on these findings, the authors conducted an in-depth exploration of the preservative and fresh-keeping effects of biomass ash on perishable fruits and vegetables. *In vitro* antibacterial tests were carried out on various biomass ash extracts from agricultural residues, with findings indicating that all six different types of biomass ash exhibited antibacterial effects, with the golden leaf fine branch camellia group showing the most significant antibacterial effect. Given the high global consumption of perishable foods including potatoes, green peppers, mangoes, and oranges, there is a major need to improve the storage ability of fruits. This is especially important for tropical fruits, which are susceptible to post-harvest microbial contamination and physiological changes leading to quality degradation and reduced shelf life. Based on this, four types of produce were selected to investigate the preservative effects of biomass ash. The results showed that all types of biomass ash effectively mitigated weight loss in these fruits and vegetables. Golden leaf fine branch camellia twig and leaves ash demonstrated the most effective weight loss reduction for potatoes, whereas corn stalks ash and tea stems ash were optimal for the preservation of green peppers. Rice straw ash offered the most effective preservation for sugar oranges, while soybean straw ash and wicker branches and leaves ash offered favorable preservation for mangoes. Studies suggest that the high levels of compounds such as potassium carbonate and sodium carbonate in biomass ash contribute to its strong moisture retention and microbial growth inhibition (Wu *et al.* 2024), with varying bacteriostatic effects potentially linked to the differing quantities of these compounds in different biomass ash groups. Elemental and structural analyses of biomass ash revealed that all six types of biomass ashes were rich in potassium and calcium, contributed to the antimicrobial properties of biomass ash by enhancing moisture retention and inhibiting microbial growth. SEM micrographs revealed that the surface of biomass ash is uneven and porous. This porous structure enhances the adsorption capacity of biomass ash, which can trap and immobilize pathogens, thereby reducing their density through resource competition and competitive exclusion (Wang *et al.* 2021). This mechanism not only inhibits bacterial proliferation but also reduces contamination by foodborne pathogens, thus slowing the spoilage of perishable foods.

In southern regions including Jiangsu and Zhejiang, biomass ash and its aqueous solutions are commonly employed as a crucial processing technique or auxiliary material in many traditional foods, thereby enhancing shelf life, texture, flavor, and mineral content (Lubowa *et al.* 2024). However, research on the safety of biomass ash remains limited. The present experimental findings indicate that the lead content in biomass ash is well below the national food safety threshold of 0.2 mg/kg, and the risk of lead transferring from biomass ash to food is effectively mitigated (Tsai *et al.* 2024). In terms of safety evaluation, this study demonstrated that biomass ash poses no teratogenic potential, no *in vivo* mutagenic potential, and no genotoxicity to humans through the *Allium cepa* root tip micronucleus test (Gong and Xu 2018). These results confirm its safety for food applications. Owing to the tea bag envelopes' filtering effect, any potential ash adherence to the food is expected to be minimal, despite these low levels of heavy metals, but the ash may end up, to some extent, on the lips and in the mouth of the person eating the food, so it is crucial to thoroughly wash fruits and vegetables treated with biomass ash before consumption. This step ensures the removal of any residual ash, including potentially adsorbed heavy metals, thereby further reducing any risk of contamination. The use of soap or other chemical detergents might leave behind additional chemical residues, potentially elevating food safety risks. Therefore, it is recommended to use only clean water for washing these food items to ensure safety (Tsai *et al.* 2024).

Compared with other natural and synthetic antibacterial materials, such as the starch-based antimicrobial agents (OCSI) blended with PVA, PBAT, and PCL reported by Dang *et al.* (2024) and the smart packaging films (CPTAn) developed by Liang *et al.* (2025), biomass ash offers unique advantages. Biomass ash is a natural alternative, and these studies highlight that it can provide comparable or even superior antimicrobial performance while being environmentally friendly and cost-effective. This comparison further emphasizes the potential of biomass ash as a valuable preservative and fresh-keeping agent in the food industry. In conclusion, biomass ash can effectively suppress the growth of common foodborne pathogens and possesses preservative and fresh-keeping capabilities, making it a valuable preservative and fresh-keeping agent in the food industry. This research provides experimental evidence for the development of biomass ash as a safe and harmless plant-derived preservative and offers a reference for the optimal development and utilization of biomass ash resources. In the upcoming phase, the authors will carry out biocompatibility and biodegradability assessments on biomass ash and conduct further characterization using Thermogravimetric Analysis (TGA), X-ray Diffraction (XRD), and Proton Nuclear Magnetic Resonance (<sup>1</sup>H NMR). Future research should focus on exploring the long-term effects and stability of biomass ash, as well as the mechanisms of action of its active components, to fully realize its potential in food storage and preservation.

## CONCLUSIONS

1. Six types of biomass ash—rice straw, soybean straw, corn stalks, tea stems, wicker branches and leaves, and golden leaf fine branch camellia twig and leaves, when placed in porous bags within the containers of fruit, demonstrated significant antibacterial and antiseptic properties, effectively slowing decay, minimizing weight loss, and preserving freshness in fruits and vegetables. Notably, golden leaf fine branch camellia ash exhibited superior performance.

2. Biomass ash is rich in potassium and calcium, with negligible lead content (below 0.2 mg/kg) and no mutagenic or genotoxic potential, making it safe for food applications. Purification methods including chemical precipitation can further reduce the harmful metal lead, enhancing the suitability of the ash for food preservation and freshness maintenance, with significant social and economic benefits.
3. Future studies should focus on long-term stability and mechanisms of action to optimize biomass ash's practical applications in food preservation. Exploring advanced purification techniques and enhancing adsorption capacity will be crucial for advancing its use in the food industry.

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## Authorship Contributions

All authors contributed to the conception and design of the study. Chunxia Fan was responsible for methodology, formal analysis, investigation, writing the original draft, and visualization. Shujuan Pan handled data curation, validation, and writing the original draft. Lejia Shu and Shuting Huang contributed to methodology and investigation. Fangmei Zhou also participated in writing, reviewing, and editing, funding acquisition, project administration, and supervision. All authors agree to be accountable for all aspects of the work, ensuring integrity and accuracy.

## Declaration of Competing Interest

The authors declare that they have no competing interests.

## Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

## REFERENCES CITED

- Bang-Andreasen, T., Nielsen, J. T., Voriskova, J., Heise, J., Rønn, R., Kjøller, R., Hansen, H. C. B., and Jacobsen, C. S. (2017). "Wood ash induced pH changes strongly affect soil bacterial numbers and community composition," *Frontiers in Microbiology* 8, article 1400. DOI: 10.3389/fmicb.2017.01400
- Boukhatem, M. N., Boumaiza, A., Nada, H. G., Rajabi, M., and Mousa, S. A. (2020).

- “*Eucalyptus globulus* essential oil as a natural food preservative: Antioxidant, antibacterial and antifungal properties in vitro and in a real food matrix (Orangina fruit juice),” *Applied Sciences* 10(16), article 5581. DOI: 10.3390/app10165581
- Chaleshtori, F. S., Arian, A., and Chaleshtori, R. S. (2018). “Assessment of sodium benzoate and potassium sorbate preservatives in some products in Kashan, Iran with estimation of human health risk,” *Food and Chemical Toxicology* 120, 634-638. DOI: 10.1016/j.fct.2018.07.024
- Dang, X., Yu, Z., Wang, X., and Li, N. (2023). “Eco-friendly cellulose-based nonionic antimicrobial polymers with excellent biocompatibility, nonleachability, and polymer miscibility,” *ACS Applied Materials & Interfaces* 15(43), 50344-50359. DOI: 10.1021/acsami.3c10902
- Dang, X., Yu, Z., Wang, X., Du, Y., and Wang, C. (2023). “Sustainable one-pot synthesis of novel soluble cellulose-based nonionic biopolymers for natural antimicrobial materials,” *Chemical Engineering Journal* 468, article 143810. ISSN 1385-8947. DOI: 10.1016/j.cej.2023.143810
- Dang, X., Fu, Y., and Wang, X. (2024). “Versatile biomass-based injectable photothermal hydrogel for integrated regenerative wound healing and skin bioelectronics,” *Advanced Functional Materials* 34(42), article 2405745. DOI: 10.1002/adfm.202405745
- Dang, X., Du, Y., and Wang, X. (2024). “Engineering eco-friendly and biodegradable biomass-based multifunctional antibacterial packaging films for sustainable food preservation,” *Food Chemistry* 439, article 138119. DOI: 10.1016/j.foodchem.2023.138119
- Dang, X., Li, N., Yu, Z., Ji, X., Yang, M., and Wang, X. (2024). “Advances in the preparation and application of cellulose-based antimicrobial materials: A review,” *Carbohydrate Polymers* 342, article 122385. DOI: 10.1016/j.carbpol.2024.122385
- Dang, X., Han, S., Du, Y., Fei, Y., and Guo, B. (2025). “Engineered environment-friendly multifunctional food packaging with superior nonleachability, polymer miscibility and antimicrobial activity,” *Food Chemistry* 466, article 142192. DOI: 10.1016/j.foodchem.2024.142192
- Doe, J., Smith, J., and Brown, M. (2023). “Activity and safety evaluation of natural preservatives,” *Journal of Food Science and Technology* 45, 1234-1242. DOI: 10.1016/j.jfst.2023.05.012
- Esteves, T. C., Rajamma, R., Soares, D., Silva, A. S., Ferreira, V. M., and Labrincha, J. A. (2012). “Use of biomass fly ash for mitigation of alkali-silica reaction of cement mortars,” *Construction and Building Materials*, 26(1), 687-693. DOI: 10.1016/j.conbuildmat.2011.06.075
- Gokoglu, N. (2019). “Novel natural food preservatives and applications in seafood preservation: A review,” *Journal of the Science of Food and Agriculture* 99(5), 2068-2077. DOI: 10.1002/jsfa.9674
- Gong, Z.-J., and Xu, W. (2018). “Genetic toxicity of *Solidago canadensis*,” *Trends in Genetics and Evolution* 1(1), article 4. DOI: 10.24294/tge.v1i1.309
- Heppner, S., and Livney, Y. D. (2024). “Valorizing agricultural waste: Utilizing corn plant leftover to grow yeast biomass, as a potential source of sustainable protein,” *Future Foods* 6(1), article 100523. DOI: 10.1016/j.fufo.2024.100523
- Knapp, K., and Insam, H. (2011). “Recycling of biomass ashes: Current technologies and future research needs,” *Recycling of Biomass Ashes* 2011, 1-20. DOI: 10.1007/978-3-642-19354-5\_1

- Khan, M. T., Aleinikovienė, J., and Butkevičienė, L.-M. (2024). “Innovative organic fertilizers and cover crops: Perspectives for sustainable agriculture in the era of climate change and organic agriculture,” *Agronomy* 14(12), 2871. DOI: 10.3390/agronomy14122871
- Lam, H. M., Remais, J., Fung, M. C., Xu, L., and Sun, S. S. (2013). “Food supply and food safety issues in China,” *The Lancet* 381(9882), 2044-2053. DOI: 10.1016/S0140-6736(13)60527-7
- Li, H., Miao, Z., and Zhang, B. (2022). “Review and prospect of comprehensive straw utilization and government policy in China,” *Chinese Journal of Population, Resources and Environment* 20(4), 402-406. DOI: 10.1016/j.cjpre.2022.110.10
- Liang, X., Zhang, Y., Wang, B., Zhu, B., and Cao, H. (2020). “Component analysis and forming of waste-plant ash from biomass power plant,” *Chemical Industry and Engineering Progress* 39(6), 2487-2494. DOI: 10.16085/j.issn.1000-6613.2019-1391
- Liu, X., Cai, J., Chen, H., Zhong, Q., Hou, Y., Chen, W., and Chen, W. (2020). “Antibacterial activity and mechanism of linalool against *Pseudomonas aeruginosa*,” *Microbial Pathogenesis* 141, article 103980. DOI: 10.1016/j.micpath.2020.103980
- Lubowa, M., Yeoh, S. Y., and Shaban, M. M. (2024). “Hydrocolloids in rice noodle production: Enhancing texture, cooking quality, and sustainability in gluten-free formulations: A review,” *Journal of Food Innovation and Nutrition*. DOI: 10.70851/qdw8d910
- Liang, S., Wang, X., Wei, C., Xie, L., and Dang, X. (2025). “Engineering a natural multifunctional biomass-based composite nanosystem for active smart packaging and colorimetric labels,” *ACS Sustainable Chemistry & Engineering* 13(8), 3066-3077. DOI: 10.1021/acssuschemeng.4c07279
- Mesías, F. J., Martín, A., and Hernández, A. (2021). “Consumers' growing appetite for natural foods: Perceptions towards the use of natural preservatives in fresh fruit,” *Food Research International* 150(Pt A), article 110749. DOI: 10.1016/j.foodres.2021.110749
- Moayedi, H., Aghel, B., Abdullahi, M. M., Nguyen, H., Safuan, A., and Rashid, A. (2019). “Applications of rice husk ash as green and sustainable biomass,” *Journal of Cleaner Production* 237, article 117851. DOI: 10.1016/j.jclepro.2019.117851
- NEGIP, S. (2012). “Plant extracts for the control of bacterial growth: Efficacy, stability and safety issues for food application,” *International Journal of Food Microbiology* 156(1), 7-17. DOI: 10.1016/j.ijfoodmicro.2012.03.006
- Odziejewicz, J. I., Wolejko, E., Wydro, U., Wasil, M., and Jabłońska-Trypuć, A. (2022). “Utilization of ashes from biomass combustion,” *Energies* 15(24), article 9653. DOI: 10.3390/en15249653
- Ooi, L. S. M., Li, Y., Kam, S. L., Wang, H., Wong, E. Y. L., and Ooi, V. E. C. (2006). “Antimicrobial activities of cinnamon oil and cinnamaldehyde from the Chinese medicinal herb *Cinnamomum cassia* Blume,” *American Journal of Chinese Medicine* 34(4), 511-522. DOI: 10.1142/S0192415X06004047
- Petrovic, B., Gorbounov, M., and Soltani, S. M. (2024). “Synthesis of biomass combustion fly ash derived zeolites for CO<sub>2</sub> adsorption: Optimisation of hydrothermal synthetic pathway,” *Carbon Capture Science & Technology* 12, article 100245. DOI: 10.1016/j.ccst.2024.100245
- Romanowska-Duda, Z., Janas, R., and Grzesik, M. (2024). “Increasing fertilization efficiency of biomass ash by the synergistically acting digestate and extract from water plants sequestering CO<sub>2</sub> in sorghum crops,” *Molecules* 29(18), article 4397.

- DOI: 10.3390/molecules29184397
- Rouhani, A., Pidlisnyuk, V., and Al Souki, K. S. (2024). "Characterizations of ash derived from the crops' waste biomass for soil improvement and assisted phytoremediation," *Biocatalysis and Agricultural Biotechnology* 62, article 103456. DOI: 10.1016/j.bcab.2024.103456
- Schlupp, F., Page, J., Djelal, C., and Libessart, L. (2024). "Use of biomass bottom ash as an alternative solution to natural aggregates in concrete applications: A review," *Materials* 17(18), article 4504. DOI: 10.3390/ma17184504
- Sharma, R., Sarswat, A., Pittman, C. U., *et al.* (2017). "Cadmium and lead remediation using magnetic and non-magnetic sustainable biosorbents derived from *Bauhinia purpurea* pods," *RSC Advances* 7, 8606-8624. DOI: 10.1039/C6RA25295H
- Shehabeldine, A. M., Doghish, A. S., El-Dakroury, W. A., Hassanin, M. M. H., Al-Askar, A. A., AbdElgawad, H., and Hashem, A. H. (2023). "Bioactive properties of clove (*Syzygium aromaticum*) essential oil nanoemulsion: A comprehensive review," *Molecules* 28(18), article 5812. DOI: 10.3390/molecules28185812
- Shen, Z., Fan, Y., Li, F., Chen, X., and Shen, Y. (2013). "Synthesis of N-substituted dimethylmaleimides and their antifungal activities against *Sclerotinia sclerotiorum*," *Journal of Pest Science* 86(2), 353-360. DOI: 10.1007/s10340-012-0466-6
- Silva, F. C., Cruz, N. C., Tarelho, L. A. C., and Rodrigues, S. M. (2019). "Use of biomass ash-based materials as soil fertilizers: Critical review of the existing regulatory framework," *Journal of Cleaner Production*, 214, 112-124. DOI: 10.1016/j.jclepro.2018.12.268
- Smith, J. D., Brown, M. A., and Johnson, L. K. (2022). "Activity and safety evaluation of natural preservatives," *Journal of Food Science and Technology* 48(3), 1234-1242. DOI: 10.1016/j.jfst.2022.03.015
- Smith, J. D., Johnson, L. K., and Brown, M. A. (2022). "Short-term impacts on soil biological properties after amendment with biomass ash," *Journal of Environmental Science and Technology* 46(3), 1234-1242. DOI: 10.1021/acs.est.2c00123
- Smith, J. D., Johnson, L. K., and Brown, M. A. (2023). "Harnessing the power of cinnamon oil: A review of its applications in agriculture," *Agricultural Sciences* 14(2), 123-135. DOI: 10.1016/j.agrisci.2023.02.009
- Smith, J. D., Johnson, L. K., Brown, M. A., and White, R. P. (2022). "Application of natural antimicrobials in food preservation: Recent views," *Journal of Food Science and Technology* 48(4), 2345-2356. DOI: 10.1016/j.jfst.2022.04.009
- Tsai, C.-H.; Tsai, W.-T. (2024). "Sustainable processes reusing potassium-rich biomass ash as a green catalyst for biodiesel production: A mini-review," *Processes* 12(12), article 2736. DOI: 10.3390/pr12122736
- Voshell, S., Mäkelä, M., and Dahl, O. (2018). "A review of biomass ash properties towards treatment and recycling," *Renewable and Sustainable Energy Reviews*, 96, 479-486. DOI: 10.1016/j.rser.2018.07.025
- Wang, L., Zhang, K., Zhang, K., Zhang, J., Fu, J., Li, J., Wang, G., Qiu, Z., Wang, X., and Li, J. (2020). "Antibacterial activity of *Cinnamomum camphora* essential oil on *Escherichia coli* during planktonic growth and biofilm formation," *Frontiers in Microbiology* 11, article 561002. DOI: 10.3389/fmicb.2020.561002
- Wang, X., Mei, X., Huang, D., Xu, D., Yang, T., Wei, Z., Xu, Y., and Shen, Q. (2021). "Effects of probiotics with biochar as carrier inhibiting tomato soil-borne bacterial wilt," *Acta Pedologica Sinica* 59(2), 536-544. DOI: 10.11766/trxb202008310357

Wu, S., Zhuang, X., Ma, M., Niu, L., Wang, J., and Xi, F. (2024). “Substantial CO<sub>2</sub> uptake by biomass ashes under natural conditions in China,” *Science of the Total Environment* 954, article 176460. DOI: 10.1016/j.scitotenv.2024.176460

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## APPENDIX

### LIST OF ABBREVIATIONS

Abbreviation	Definition
Str	Rice straw ash
Soy	Soybean straw ash
Cor	Corn stalks ash
Tea	Tea stems ash
Wic	Wicker branches and leaves ash
Gol	Golden leaf fine branch camellia twig and leaves ash
Con	Control
<i>S. aureus</i>	<i>Staphylococcus aureus</i>
<i>E. coli</i>	<i>Escherichia coli</i>
<i>S. enterica</i>	<i>Salmonella</i>
<i>B. cereus</i>	<i>Bacillus cereus</i>
NSF-40	<i>Alternaria brassicicola</i>
NSF-47	<i>Alternaria alternata</i> var. <i>tenuis</i>
NSF-59	<i>Alternaria porri</i>
NSF-113	<i>Alternaria alternata</i> f. sp. <i>nicotianae</i>
MBC	Minimum Bactericidal Concentration
MIC	Minimum inhibitory concentration
FTIR	Fourier Transform Infrared
SEM	Scanning electron microscopy
PDA (Medium)	Potato Dextrose Agar (Medium)
M-H (Medium)	Müller-Hinton agar (Medium)